

DAFTAR PUSTAKA

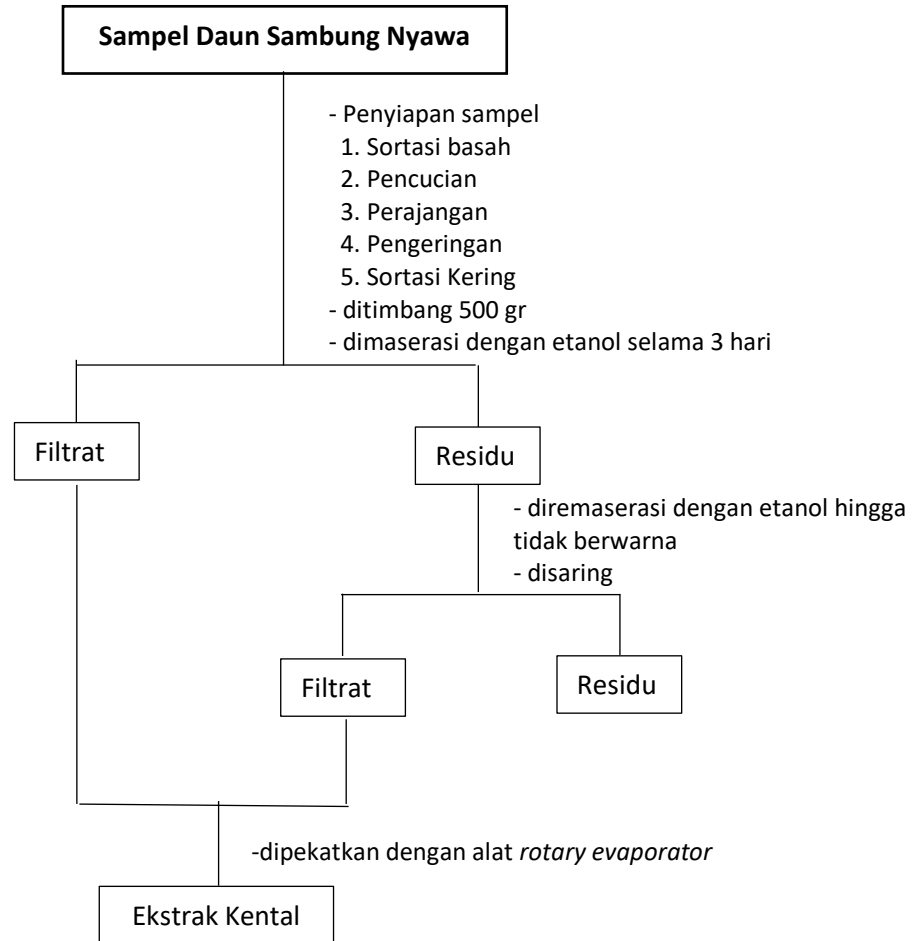
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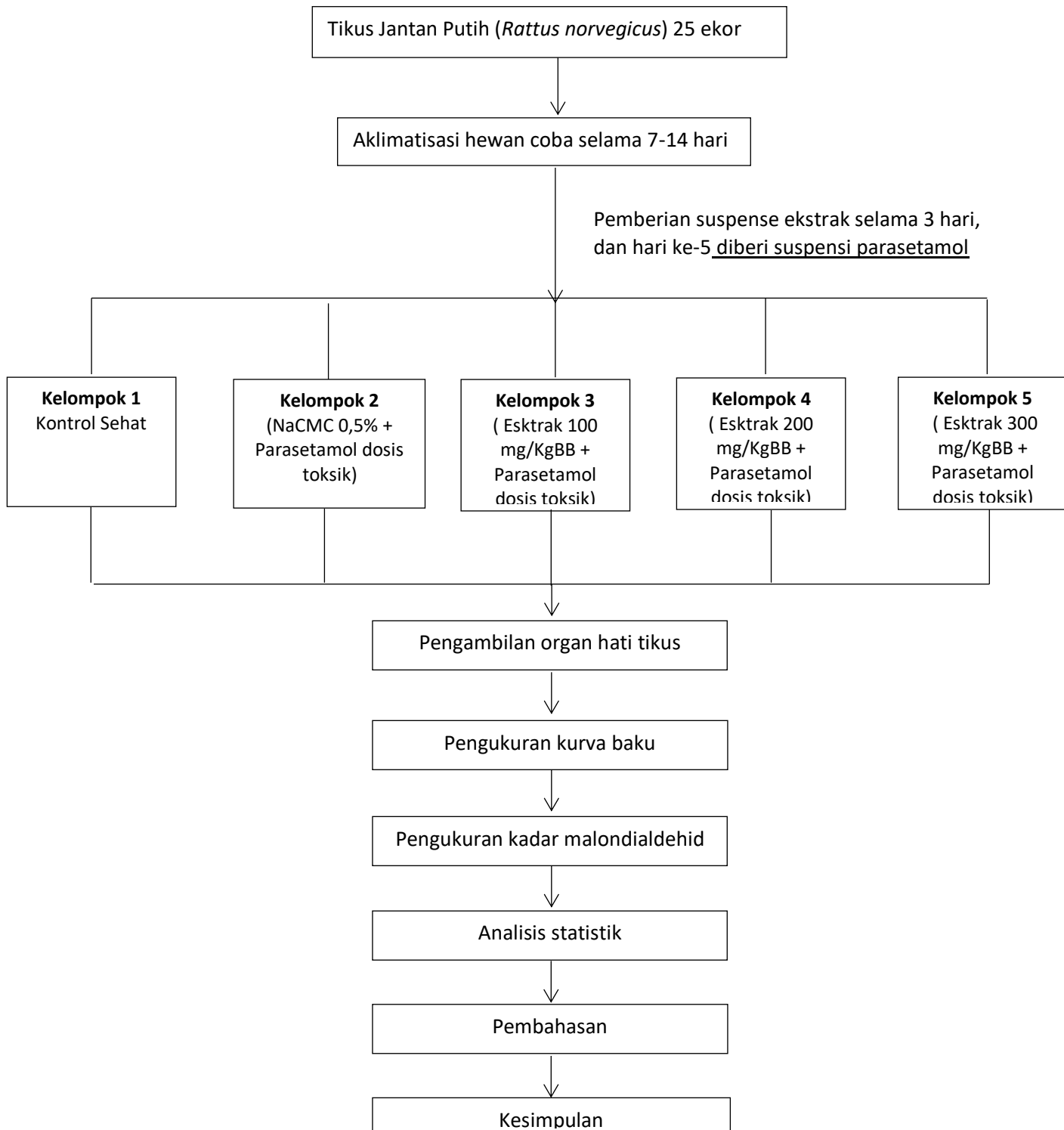
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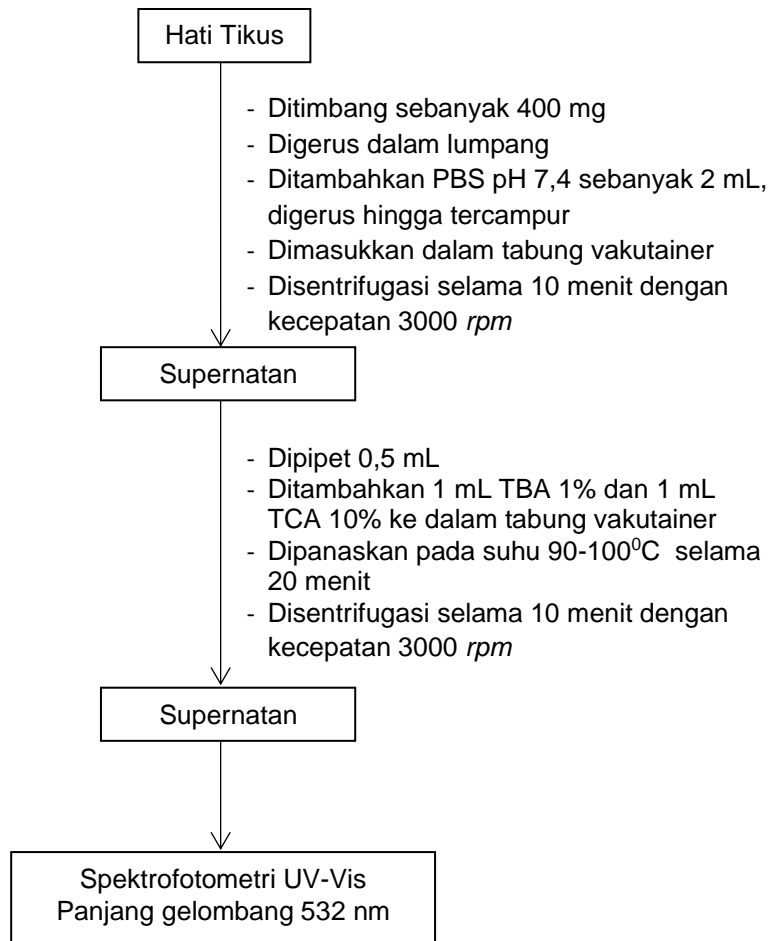
LAMPIRAN

Lampiran 1. Skema Kerja Pembuatan Ekstrak

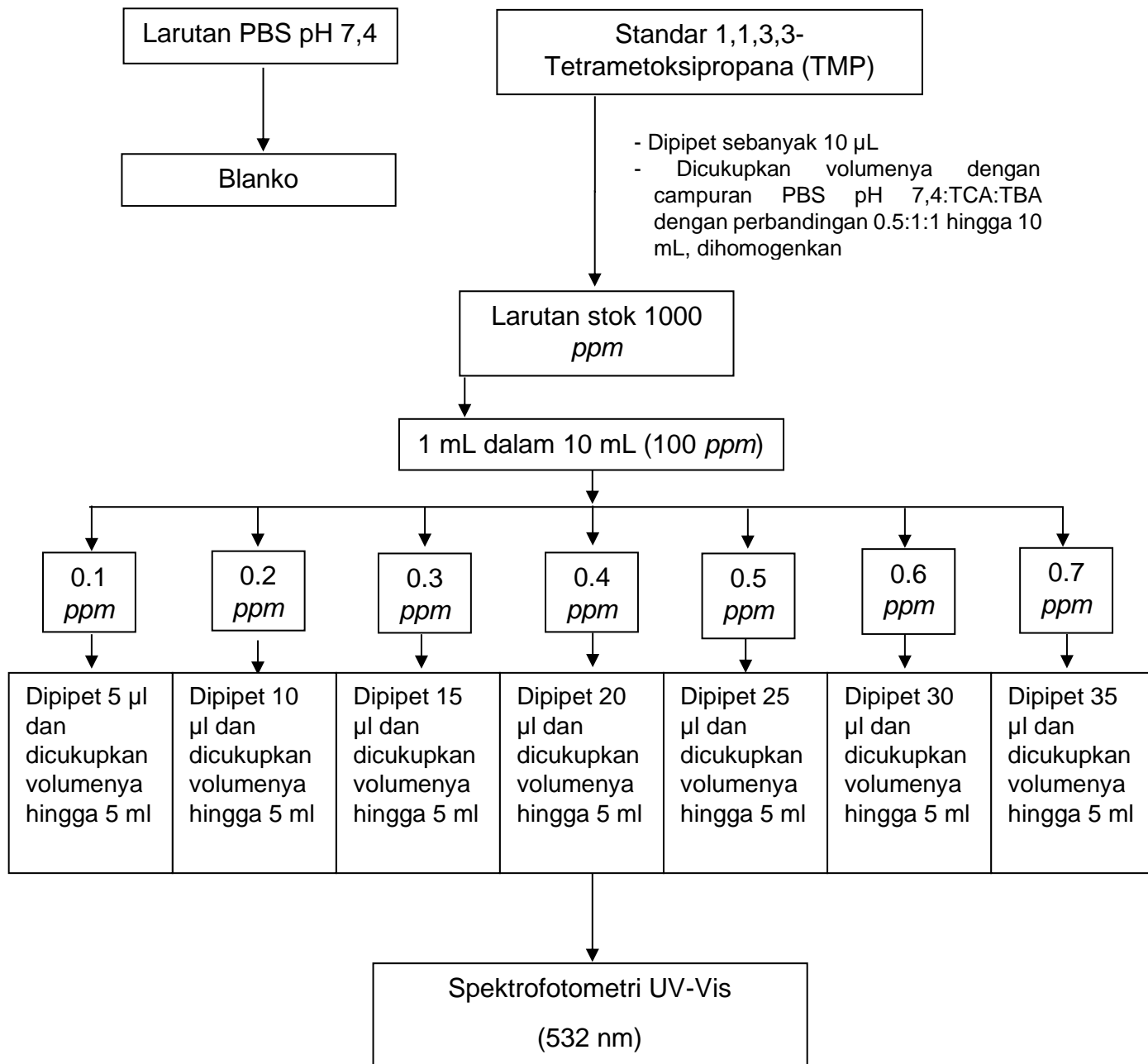


Lampiran 2. Skema Kerja Perlakuan Hewan Coba



Lampiran 3. Skema Kerja Pengukuran Kadar Malondialdehid

Lampiran 4. Skema Kerja Pengukuran Kurva Baku



Lampiran 5. Perhitungan Dosis

5.1 Parasetamol

Dosis parasetamol yang bersifat toksik pada hewan coba tikus adalah 2.400 mg/kg Berat Badan (BB) (BPOM,2006).

Untuk dosis 2.400 mg / 1000 g

Untuk BB tikus 200 g = 480 mg / 200 g /2 mL

Dibuat dalam 25 mL = 480 mg / 2 mL

= 6.000 mg / 25 mL

Jumlah serbuk tablet parasetamol yang ditimbang

$$\begin{aligned}
 &= \frac{\text{Jumlah yang diinginkan}}{\text{Berat etiket}} \times \text{berat rata – rata 20 tablet} \\
 &= \frac{6.000 \text{ mg}}{500 \text{ mg}} \times 555.56 \text{ mg} \\
 &= 6.666,72 \text{ mg}
 \end{aligned}$$

Ditimbang serbuk tablet sebanyak 6.666,72 mg kemudian disuspensikan dalam 25 mL NaCMC 0,5%. Dengan demikian, untuk membuat dalam volume 50 mL maka ditimbang 13.333,44 mg kemudian disuspensikan dalam 50 mL NaCMC 0,5%.

5.2 Ekstrak Daun Sambung Nyawa

Untuk dosis 100 mg/kgBB

100 mg / 1.000 g BB

25 mg / 250 g BB / 2 mL

Dibuat dalam 50 mL = 25 mg / 2 mL

= 625 mg / 50 mL

Ditimbang ekstrak sebanyak 625 mg kemudian disuspensikan dalam 50 mL

NaCMC 0,5%

Untuk dosis 200 mg/kgBB

200 mg / 1.000 g BB

50 mg / 250 g BB / 2 mL

Dibuat dalam 50 mL = 50 mg / 2 mL

= 1.250 mg / 50 mL

Ditimbang ekstrak sebanyak 1.250 mg kemudian disuspensikan dalam 50 mL

NaCMC 0,5%

Untuk dosis 300 mg/kgBB

300 mg / 1.000 g BB

75 mg / 250 g BB / 2 mL

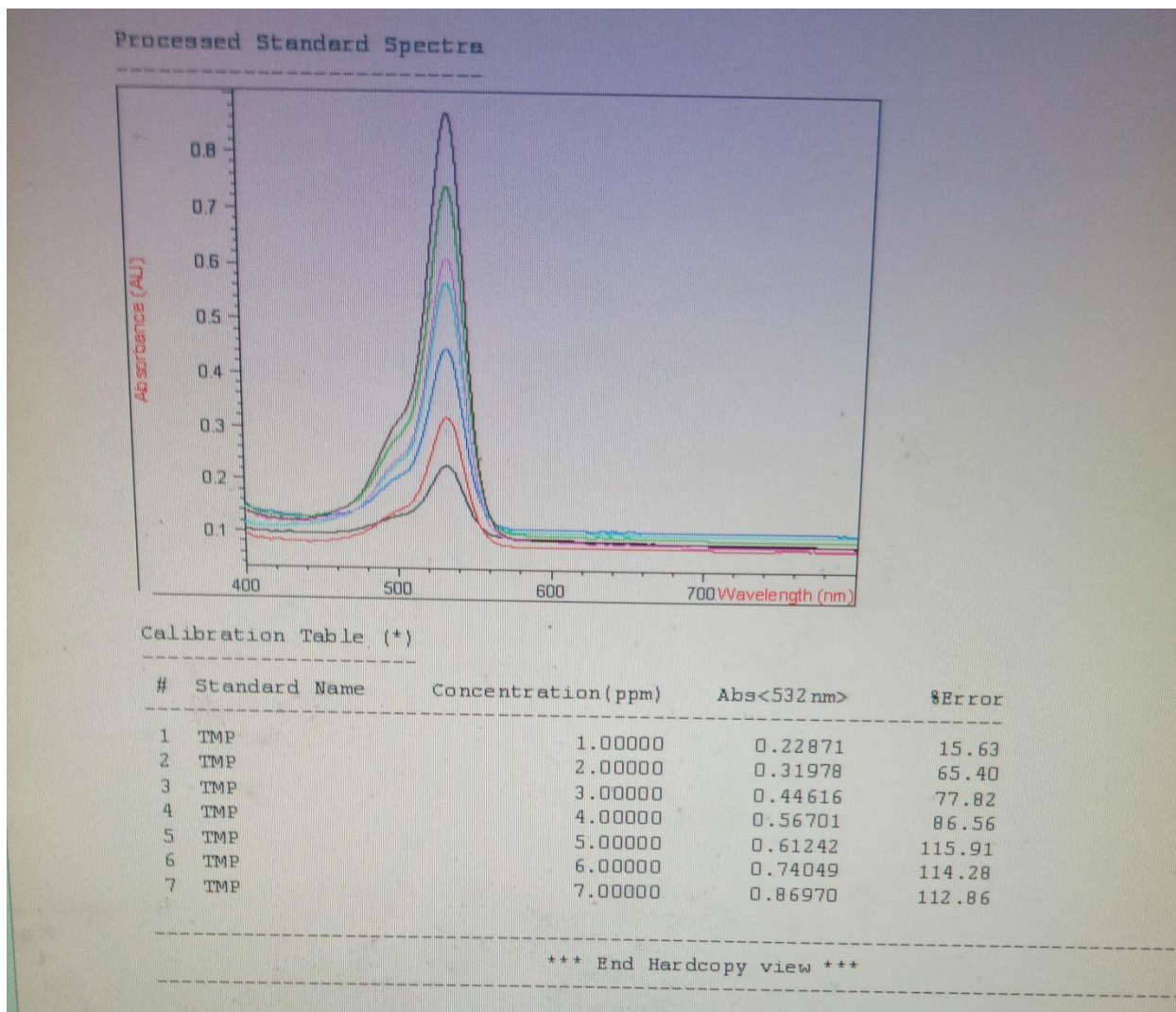
Dibuat dalam 50 mL = 75 mg / 2 mL

= 1.875 mg / 50 mL

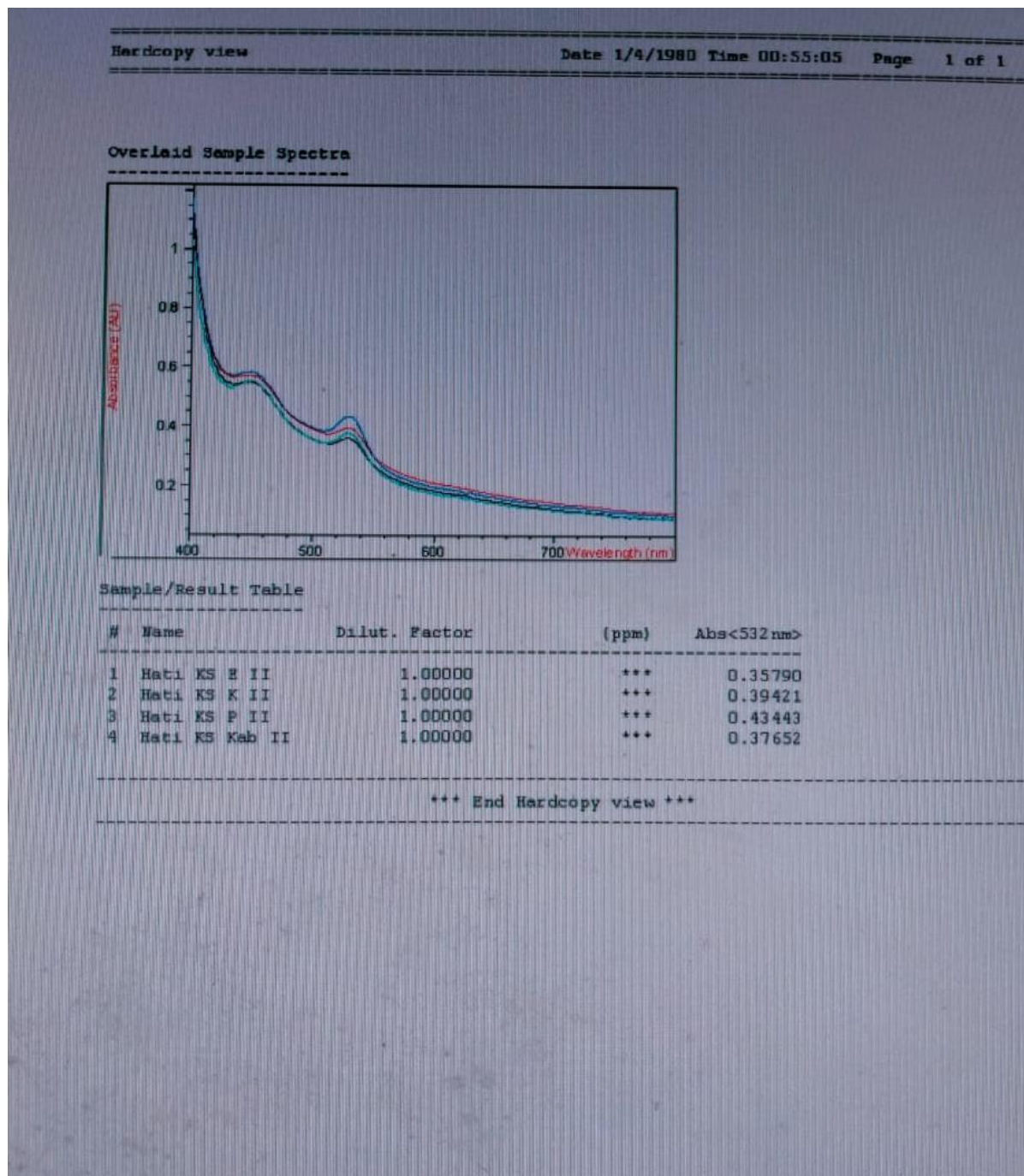
Ditimbang ekstrak sebanyak 1.875 mg kemudian disuspensikan dalam 50 mL

NaCMC 0,5%

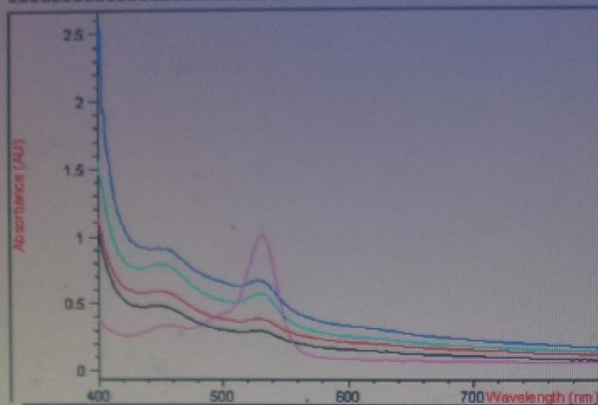
Lampiran 6. Hasil Pengukuran Absorbansi Kurva Baku



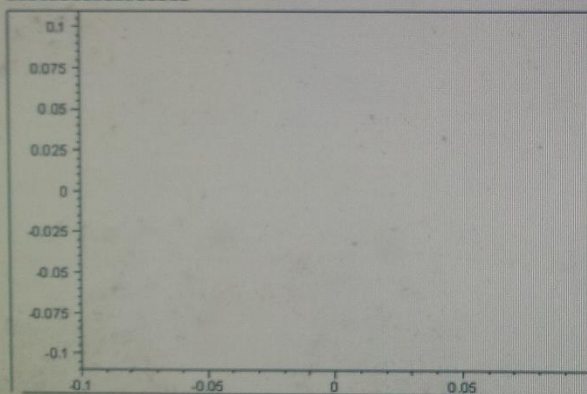
Lampiran 7. Hasil Pengukuran Absorbansi Tiap Kelompok Perlakuan



Processed Standard Spectra



Calibration Curve



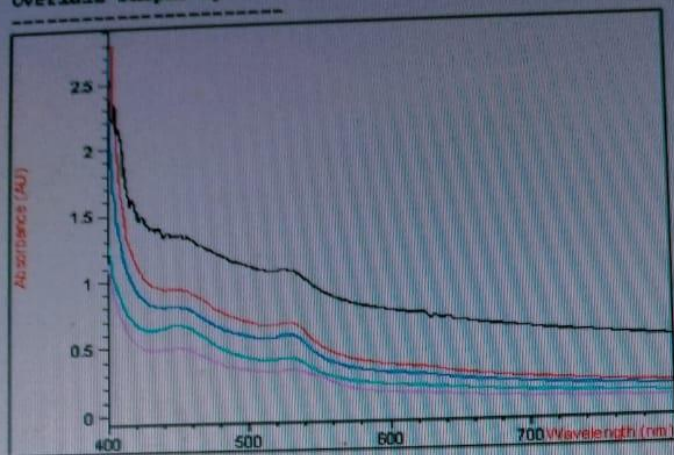
Calibration Table (*)

#	Standard Name	Concentration(ppm)	Abs<532 nm>	%Error
1	Hati NaCMC E I N	0.00000	0.29314	***
2	Hati NaCMC K I N	0.00000	0.38027	***
3	Hati NaCMC Kab I	0.00000	0.66716	***
4	Hati NaCMC Kad I	0.00000	0.57466	***
5	Hati NaCMC P I N	0.00000	1.00830	***

Hardcopy view

Date 1/4/1980 Time 00:42:24 Page 1 of

Overlaid Sample Spectra



Sample/Result Table

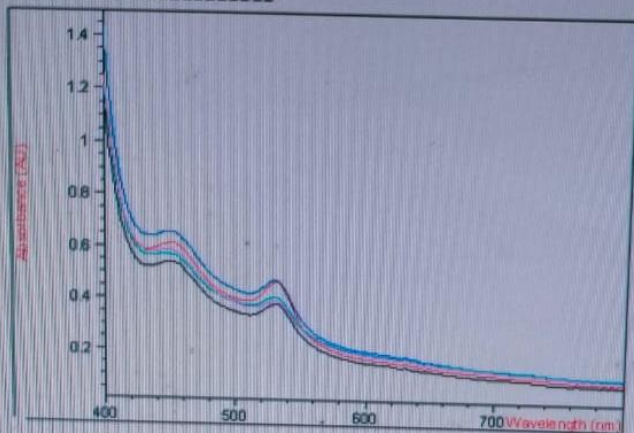
#	Name	Dilut. Factor	(ppm)	Abs<532nm>
1	Hati D 100 Kab I	1.00000	***	1.05180
2	Hati D 100 Kad I	1.00000	***	0.66465
3	Hati D 100 E III	1.00000	***	0.58017
4	Hati D 100 K III	1.00000	***	0.40588
5	Hati D 100 P III	1.00000	***	0.31863

*** End Hardcopy view ***

Hardcopy view

Date 1/4/1980 Time 02:40:41 Page 1 of 1

Overlaid Sample Spectra

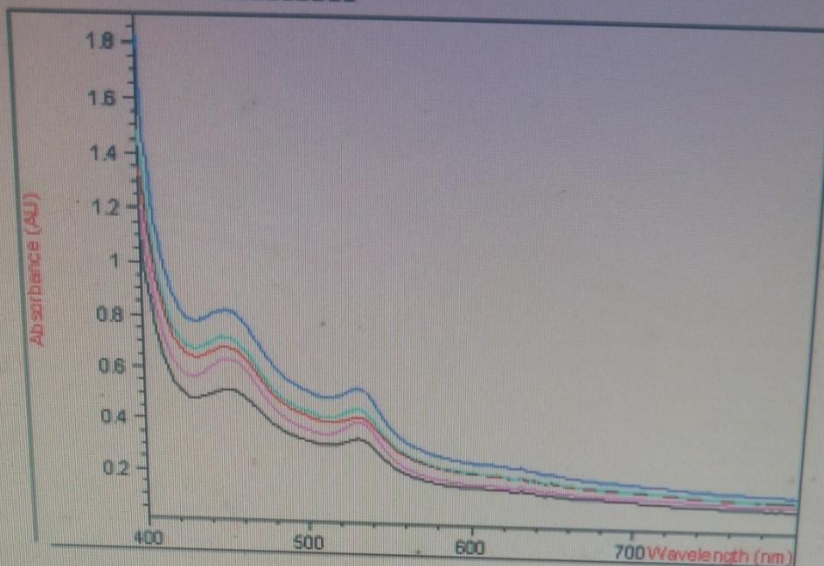


Sample/Result Table

#	Name	Dilut. Factor	(ppm)	Abs<532nm>
1	Hati D 200 Kab I	1.00000	***	0.38348
2	Hati D 200 Ked I	1.00000	***	0.46916
3	Hati D 200 P IV	1.00000	***	0.47414
4	Hati D 200 E IV	1.00000	***	0.40852
5	Hati D 200 K IV	1.00000	***	0.39272

*** End Hardcopy view ***

Overlaid Sample Spectra



Sample/Result Table

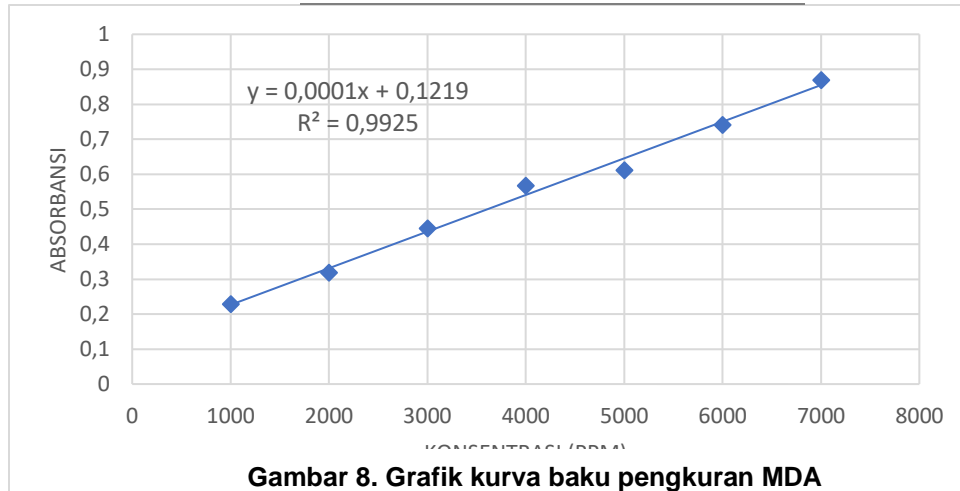
#	Name	Dilut. Factor	(ppm)	Abs<532 nm>
1	Hati D 300 K V	1.00000	***	0.33658
2	Hati D 300 P V	1.00000	***	0.42192
3	Hati D 300 Kad V	1.00000	***	0.53284
4	Hati D 300 E V	1.00000	***	0.45282
5	Hati D 300 Kab V	1.00000	***	0.40269

*** End Hardcopy view ***

Lampiran 8. Grafik Kurva Standar

Tabel 3. Absorbansi standar MDA pada konsentrasi 0.1-0.7 ppm

Konsentrasi (ppm)	Absorbansi
0,1	0,22871
0,2	0,31978
0,3	0,44616
0,4	0,56701
0,5	0,61242
0,6	0,74099
0,7	0,86970



Lampiran 9. Perhitungan Nilai X dan Kadar MDA**1. Perhitungan Nilai X**

Persamaan garis kurva baku;

$$Y = 0,0001x + 0,1219$$

Kelompok I : Kelompok kontrol**KS E II**

$$0,35790 = 0,0001x + 0,1219$$

$$x = \frac{0,35790 - 0,1219}{0,0001}$$

$$x = 2.360$$

KS K II

$$0,39421 = 0,0001x + 0,1219$$

$$x = \frac{0,39421 - 0,1219}{0,0001}$$

$$x = 2.723,1$$

KS P II

$$0,43443 = 0,0001x + 0,1219$$

$$x = \frac{0,43443 - 0,1219}{0,0001}$$

$$x = 3.125,3$$

KS Kab II

$$0,37652 = 0,0001x + 0,1219$$

$$x = \frac{0,37652 - 0,1219}{0,0001}$$

$$x = 2.546,2$$

Kelompok II : Suspensi NaCMC 0,5% + Suspensi Parasetamol**NaCMC E I N**

$$0,29314 = 0,0001x + 0,1219$$

$$x = \frac{0,29314 - 0,1219}{0,0001}$$

$$x = 1.713,4$$

NaCMC K I N

$$0,38027 = 0,0001x + 0,1219$$

$$x = \frac{0,38027 - 0,1219}{0,0001}$$

$$x = 2.583,7$$

NaCMC P I N

$$1,00830 = 0,0001x + 0,1219$$

$$x = \frac{1,00830 - 0,1219}{0,0001}$$

$$x = 8.864$$

NaCMC Kad I N

$$0,57466 = 0,0001x + 0,1219$$

$$x = \frac{0,57466 - 0,1219}{0,0001}$$

$$x = 4.527,6$$

NaCMC Kab I N

$$0,66716 = 0,0001x + 0,1219$$

$$x = \frac{0,66716 - 0,1219}{0,0001}$$

$$x = 5.452,6$$

**Kelompok III : Ekstrak Daun Sambung Nyawa 100 mg/KgBB
D 100 E III**

$$0,58017 = 0,0001x + 0,1219$$

$$x = \frac{0,58017 - 0,1219}{0,0001}$$

$$x = 4.582,7$$

D 100 K III

$$0,40588 = 0,0001x + 0,1219$$

$$x = \frac{0,40588 - 0,1219}{0,0001}$$

$$x = 2.839,8$$

D 100 P III

$$0,31863 = 0,0001x + 0,1219$$

$$x = \frac{0,31863 - 0,1219}{0,0001}$$

$$x = 1.967,3$$

D 100 Kad III

$$0,66465 = 0,0001x + 0,1219$$

$$x = \frac{0,66465 - 0,1219}{0,0001}$$

$$x = 5.427,5$$

D 100 Kab III

$$1,05180 = 0,0001x + 0,1219$$

$$x = \frac{1,05180 - 0,1219}{0,0001}$$

$$x = 9.299$$

Kelompok IV : Ekstrak Daun Sambung Nyawa 200 mg/KgBB**D 200 E IV**

$$0,40852 = 0,0001x + 0,1219$$

$$x = \frac{0,40852 - 0,1219}{0,0001}$$

$$x = 2.866,2$$

D 200 K IV

$$0,39272 = 0,0001x + 0,1219$$

$$x = \frac{0,39272 - 0,1219}{0,0001}$$

$$x = 2.708,2$$

D 200 P IV

$$0,47414 = 0,0001x + 0,1219$$

$$x = \frac{0,47414 - 0,1219}{0,0001}$$

$$x = 3.522,4$$

D 200 Kad IV

$$0,46916 = 0,0001x + 0,1219$$

$$x = \frac{0,46916 - 0,1219}{0,0001}$$

$$x = 3.472,6$$

D 200 Kab IV

$$0,38348 = 0,0001x + 0,1219$$

$$x = \frac{0,38348 - 0,1219}{0,0001}$$

$$x = 2.615,8$$

Kelompok V : Ekstrak Daun Sambung Nyawa 300 mg/KgBB**D 300 E V**

$$0,45282 = 0,0001x + 0,1219$$

$$x = \frac{0,45282 - 0,1219}{0,0001}$$

$$x = 3.309,2$$

D 300 K V

$$0,33658 = 0,0001x + 0,1219$$

$$x = \frac{0,33658 - 0,1219}{0,0001}$$

$$x = 2.146,8$$

D 300 P V

$$0,42192 = 0,0001x + 0,1219$$

$$x = \frac{0,42192 - 0,1219}{0,0001}$$

$$x = 3.000,2$$

D 300 Kad V

$$0,53284 = 0,0001x + 0,1219$$

$$x = \frac{0,53284 - 0,1219}{0,0001}$$

$$x = 4.109,4$$

D 300 Kab V

$$0,40269 = 0,0001x + 0,1219$$

$$x = \frac{0,40269 - 0,1219}{0,0001}$$

$$x = 2.807,9$$

2. Perhitungan kadar MDA

Kadar MDA dihitung dengan menggunakan rumus :

$$\text{Kadar MDA} = (x) \times D$$

Ket : x = Hasil perhitungan nilai absorbansi sampel dengan persamaan kurva baku

D = Faktor Pengenceran

Kelompok I : Kelompok kontrol

KS E II

$$\begin{aligned}\text{Kadar MDA} &= 2.360 \times 0,2 \\ &= 472 \mu\text{g/ml} \\ &= 472.000 \text{ ng/ml}\end{aligned}$$

KS K II

$$\begin{aligned}\text{Kadar MDA} &= 2.723,1 \times 0,2 \\ &= 544,62 \mu\text{g/ml} \\ &= 544.620 \text{ ng/ml}\end{aligned}$$

KS P II

$$\begin{aligned}\text{Kadar MDA} &= 3.125,3 \times 0,2 \\ &= 625,06 \mu\text{g/ml} \\ &= 625.060 \text{ ng/ml}\end{aligned}$$

KS Kab II

$$\begin{aligned}\text{Kadar MDA} &= 2.546 \times 0,2 \\ &= 509,2 \mu\text{g/ml} \\ &= 509.200 \text{ ng/ml}\end{aligned}$$

Kelompok II : Suspensi NaCMC 1% + Suspensi Parasetamol**NaCMC E I N**

$$\begin{aligned}\text{Kadar MDA} &= 1.713,4 \times 0,2 \\ &= 342,68 \mu\text{g/ml} \\ &= 342.680 \text{ ng/ml}\end{aligned}$$

NaCMC K I N

$$\begin{aligned}\text{Kadar MDA} &= 2.583,7 \times 0,2 \\ &= 516,74 \mu\text{g/ml} \\ &= 516.740 \text{ ng/ml}\end{aligned}$$

NaCMC P I N

$$\begin{aligned}\text{Kadar MDA} &= 8.864 \times 0,2 \\ &= 1.772,8 \mu\text{g/ml} \\ &= 1.772.800 \text{ ng/ml}\end{aligned}$$

NaCMC Kad I N

$$\begin{aligned}\text{Kadar MDA} &= 4.527,6 \times 0,2 \\ &= 905,52 \mu\text{g/ml} \\ &= 905.520 \text{ ng/ml}\end{aligned}$$

NaCMC Kab I N

$$\begin{aligned}\text{Kadar MDA} &= 5.452,6 \times 0,2 \\ &= 1.090,52 \mu\text{g/ml} \\ &= 1.090.520 \text{ ng/ml}\end{aligned}$$

Kelompok III : Ekstrak Daun Sambung Nyawa 100 mg/KgBB**D 100 E III**

$$\begin{aligned}\text{Kadar MDA} &= 4.582,7 \times 0,2 \\ &= 916,54 \mu\text{g/ml} \\ &= 916.540 \text{ ng/ml}\end{aligned}$$

D 100 K III

$$\begin{aligned}\text{Kadar MDA} &= 2.839,8 \times 0,2 \\ &= 567,96 \mu\text{g/ml} \\ &= 567.960 \text{ ng/ml}\end{aligned}$$

D 100 P III

$$\begin{aligned}\text{Kadar MDA} &= 1.967 \times 0,2 \\ &= 393,4 \mu\text{g/ml} \\ &= 393.400 \text{ ng/ml}\end{aligned}$$

D 100 Kad III

$$\begin{aligned}\text{Kadar MDA} &= 5.427,5 \times 0,2 \\ &= 1.085,5 \mu\text{g/ml} \\ &= 1.085.500 \text{ ng/ml}\end{aligned}$$

D 100 Kab III

$$\begin{aligned}\text{Kadar MDA} &= 9.299 \times 0,2 \\ &= 1.859,8 \mu\text{g/ml} \\ &= 1.859.800 \text{ ng/ml}\end{aligned}$$

Kelompok IV : Ekstrak Daun Sambung Nyawa 200 mg/KgBB**D 200 E IV**

$$\begin{aligned}\text{Kadar MDA} &= 2.866,2 \times 0,2 \\ &= 573,24 \mu\text{g/ml} \\ &= 573.240 \text{ ng/ml}\end{aligned}$$

D 200 K IV

$$\begin{aligned}\text{Kadar MDA} &= 2.708,2 \times 0,2 \\ &= 541,64 \mu\text{g/ml} \\ &= 541.640 \text{ ng/ml}\end{aligned}$$

D 200 P IV

$$\begin{aligned}\text{Kadar MDA} &= 3.522,4 \times 0,2 \\ &= 704,48 \mu\text{g/ml} \\ &= 704.480 \text{ ng/ml}\end{aligned}$$

D 200 Kad IV

$$\begin{aligned}\text{Kadar MDA} &= 3.472,6 \times 0,2 \\ &= 694,52 \mu\text{g/ml} \\ &= 694.520 \text{ ng/ml}\end{aligned}$$

D 200 Kab IV

$$\begin{aligned}\text{Kadar MDA} &= 2.615,8 \times 0,2 \\ &= 523,16 \mu\text{g/ml} \\ &= 523.160 \text{ ng/ml}\end{aligned}$$

Kelompok V : Ekstrak Daun Sambung Nyawa 200 mg/KgBB**D 300 E V**

$$\begin{aligned}\text{Kadar MDA} &= 3.309,2 \times 0,2 \\ &= 661,84 \mu\text{g/ml} \\ &= 661.840 \text{ ng/ml}\end{aligned}$$

D 200 K V

$$\begin{aligned}\text{Kadar MDA} &= 2.164,8 \times 0,2 \\ &= 432,96 \mu\text{g/ml} \\ &= 432.960 \text{ ng/ml}\end{aligned}$$

D 200 P V

$$\begin{aligned}\text{Kadar MDA} &= 3.000,2 \times 0,2 \\ &= 600,04 \mu\text{g/ml} \\ &= 600.040 \text{ ng/ml}\end{aligned}$$

D 200 Kad V

$$\begin{aligned}\text{Kadar MDA} &= 4.109,4 \times 0,2 \\ &= 821,88 \mu\text{g/ml} \\ &= 821.880 \text{ ng/ml}\end{aligned}$$

D 200 Kab V

$$\begin{aligned}\text{Kadar MDA} &= 2.807,9 \times 0,2 \\ &= 561,58 \mu\text{g/ml} \\ &= 561.580 \text{ ng/ml}\end{aligned}$$

Lampiran 10. Hasil Analisis Statistik

Tabel 4. Hasil analisis statistik distribusi sampel menggunakan metode shapiro-wilk

		Tests of Normality		
		Shapiro-Wilk		
	Kelompok_Perlakuan	Statistic	df	Sig.
Kadar_MDA	Kelompok kontrol	,963	4	,800
	NaCMC 0,5%	,966	4	,816
	Ekstrak 100 mg/KgBB	,938	4	,645
	Ekstrak 200 mg/KgBB	,849	4	,224
	Ekstrak 300 mg/KgBB	,955	4	,749

a. Lilliefors Significance Correction

Test distribution is Normal.

Calculated from data.

Tabel 5. Hasil analisis statistik deskriptif sampel

		Descriptives						
		95% Confidence Interval for Mean						
	N	Mean	Std. Deviation	Std. Error	Lower Bound	Upper Bound	Minimum	Maximum
Kelompok kontrol	4	537,7200	65,34114	32,67057	433,7477	641,6923	472,00	625,06
NaCMC 0,5%	4	1071,3950	525,19565	262,59783	235,6915	1907,0985	516,74	1772,80
Ekstrak 100mg/KgBB	4	1107,4500	545,89633	272,94817	238,8071	1976,0929	567,96	1859,80
Ekstrak 200mg/KgBB	4	583,1400	77,07867	38,53934	460,4906	705,7894	523,16	694,52
Ekstrak 300mg/KgBB	4	564,1300	96,69254	48,34627	410,2706	717,9894	432,96	661,84
Total	20	772,7670	405,48120	90,66835	582,9960	962,5380	432,96	1859,80

Tabel 6. Hasil analisis data menggunakan metode *One Way Anova*

ANOVA					
Kadar_MDA					
	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1343705,128	4	335926,282	2,831	,062
Within Groups	1780179,940	15	118678,663		
Total	3123885,068	19			

Tabel 7. Hasil analisis statistik *Post Hoc Test*

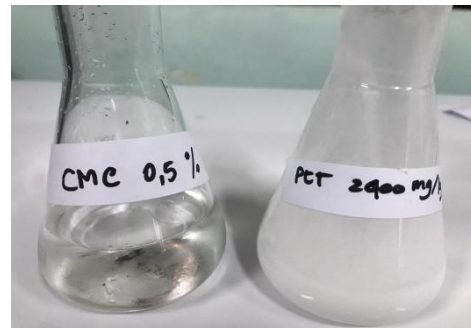
Multiple Comparisons						
Dependent Variable: Kadar_MDA						
Tukey HSD						
(I)	(J)	Mean Difference (I-	Std. Error	Sig.	95% Confidence Interval	
Kelompok_Perlakuan	Kelompok_Perlakuan	J)			Lower Bound	Upper Bound
Kelompok kontrol	NaCMC 0,5%	-533,67500	243,59666	,235	-1285,8831	218,5331
	Ekstrak 100mg/KgBB	-569,73000	243,59666	,186	-1321,9381	182,4781
	Ekstrak 200mg/KgBB	-45,42000	243,59666	1,000	-797,6281	706,7881
	Ekstrak 300mg/KgBB	-26,41000	243,59666	1,000	-778,6181	725,7981
NaCMC 0,5%	Kelompok kontrol	533,67500	243,59666	,235	-218,5331	1285,8831
	Ekstrak 100mg/KgBB	-36,05500	243,59666	1,000	-788,2631	716,1531
	Ekstrak 200mg/KgBB	488,25500	243,59666	,310	-263,9531	1240,4631
	Ekstrak 300mg/KgBB	507,26500	243,59666	,277	-244,9431	1259,4731
Ekstrak 100mg/KgBB	Kelompok kontrol	569,73000	243,59666	,186	-182,4781	1321,9381
	NaCMC 0,5%	36,05500	243,59666	1,000	-716,1531	788,2631
	Ekstrak 200mg/KgBB	524,31000	243,59666	,250	-227,8981	1276,5181
	Ekstrak 300mg/KgBB	543,32000	243,59666	,221	-208,8881	1295,5281
Ekstrak 200mg/KgBB	Kelompok kontrol	45,42000	243,59666	1,000	-706,7881	797,6281
	NaCMC 0,5%	-488,25500	243,59666	,310	-1240,4631	263,9531

	Ekstrak 100mg/KgBB	-524,31000	243,59666	,250	-1276,5181	227,8981
	Ekstrak 300mg/KgBB	19,01000	243,59666	1,000	-733,1981	771,2181
Ekstrak 300mg/KgBB	Kelompok kontrol	26,41000	243,59666	1,000	-725,7981	778,6181
	NaCMC 0,5%	-507,26500	243,59666	,277	-1259,4731	244,9431
	Ekstrak 100mg/KgBB	-543,32000	243,59666	,221	-1295,5281	208,8881
	Ekstrak 200mg/KgBB	-19,01000	243,59666	1,000	-771,2181	733,1981

Lampiran 11. Dokumentasi Penelitian



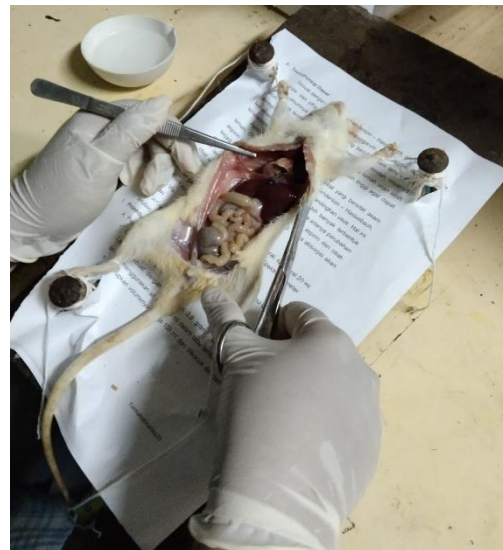
Gambar 9. Ekstrak etanol daun sambung nyawa



Gambar 10. Suspensi NaCMC 0,5% dan Suspensi Parasetamol dosis



Gambar 11. Pemberian larutan uji secara peroral



Gambar 12. Pembedahan hewan coba tikus



Gambar 13. Pembekuan organ hati menggunakan nitrogen cair



Gambar 14. Penimbangan organ hati



Gambar 15. Penggerusan organ hati



Gambar 16. Penambahan larutan uji




Gambar 17. Penyiapan Proses sentrifugasi



Gambar 18. Pengukuran kadar MDA menggunakan spektrofotometri UV-Vis

Lampiran 12. Persetujuan Kode Etik


KEMENTERIAN PENDIDIKAN, KEBUDAYAAN, RISET DAN TEKNOLOGI
UNIVERSITAS HASANUDDIN FAKULTAS KEDOKTERAN
KOMITE ETIK PENELITIAN UNIVERSITAS HASANUDDIN
RSPTN UNIVERSITAS HASANUDDIN
RSUP Dr. WAHIDIN SUDIROHUSODO MAKASSAR
 Sekretariat : Lantai 2 Gedung Laboratorium Terpadu
 JL PERINTIS KEMERDEKAAN KAMPUS TAMALANREA KM.10 MAKASSAR 90245.
 Contact Person: dr. Agussalim Bukhari.,M.Med.,Ph.D.,SpGK TELP. 081241850858, 0411 5780103, Fax : 0411-581431




REKOMENDASI PERSETUJUAN ETIK

Nomor : 761/UN4.6.4.5.31/ PP36/ 2021

Tanggal: 2 Desember 2021

Dengan ini Menyatakan bahwa Protokol dan Dokumen yang Berhubungan Dengan Protokol berikut ini telah mendapatkan Persetujuan Etik :

No Protokol	UH21100646	No Sponsor	
Peneliti Utama	Putri Alifyani	Sponsor	
Judul Peneliti	Uji Aktivitas Ekstrak Etanol Daun Sambung Nyawa (Gynura Procumbens) Terhadap Kadar Malondialdehid (Mda) Hati Tikus Putih (Rattus Norvegicus) Yang Diinduksi Paracetamol Dosis Toksik		
No Versi Protokol	1	Tanggal Versi	19 Oktober 2021
No Versi PSP		Tanggal Versi	
Tempat Penelitian	Laboratorium Fakultas Farmasi Universitas Hasanuddin Makassar		
Jenis Review	<input type="checkbox"/> Exempted <input checked="" type="checkbox"/> Expedited <input type="checkbox"/> Fullboard Tanggal	Masa Berlaku 2 Desember 2021 sampai 2 Desember 2022	Frekuensi review lanjutan
Ketua KEP Universitas Hasanuddin	Nama Prof.Dr.dr. Suryani As'ad, M.Sc.,Sp.GK (K)	Tanda tangan 	
Sekretaris KEP Universitas Hasanuddin	Nama dr. Agussalim Bukhari, M.Med.,Ph.D.,Sp.GK (K)	Tanda tangan 	

Kewajiban Peneliti Utama:

- Menyerahkan Amandemen Protokol untuk persetujuan sebelum di implementasikan
- Menyerahkan Laporan SAE ke Komisi Etik dalam 24 Jam dan dilengkapi dalam 7 hari dan Lapo SUSAR dalam 72 Jam setelah Peneliti Utama menerima laporan
- Menyerahkan Laporan Kemajuan (progress report) setiap 6 bulan untuk penelitian resiko tinggi dan setiap setahun untuk penelitian resiko rendah
- Menyerahkan laporan akhir setelah Penelitian berakhir
- Melaporkan penyimpangan dari prokol yang disetujui (protocol deviation / violation)
- Mematuhi semua peraturan yang ditentukan